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Modelling of a continuous two-phase partitioning bioreactor for the degradation of xenobiotics

Susan M. Cruickshank, Andrew J. Daugulis *, P. James McLellan

Department of Chemical Engineering, Queen's University, Kingston, Ont., Canada K7L 3N6

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Abstract

The possibility of operating a two-phase partitioning bioreactor for phenol degradation in continuous mode has been explored in this paper, using a mechanistic model for the bioreactor. Characteristics of continuous culture fermentations with substrate inhibition were investigated using steady-state and dynamic simulations. The simulations confirmed that over certain ranges of aqueous dilution rates, up to three steady states for the cell and substrate concentrations could be obtained. These corresponded to stable and unstable steady states, as well as washout conditions. The unstable nature of the steady states was examined for two different operating regions representing extremes of solvent dilution rate and feed substrate concentration. Step decreases in feed substrate concentration, when operating at the unstable steady state, resulted in the system reaching a stable steady state, while step increases in feed substrate concentration eventually resulted in the system washing out. Dynamic simulations also revealed variations in the sensitivity of the continuous model to substrate concentrations, depending on the operating point. This variation indicates non-linearity in dynamic behaviour.

Keywords: Phenol degradation; Continuous culture; Two-phase partitioning bioreactor; Dynamic modelling; Steady-state modelling

1. Introduction

The two-phase partitioning bioreactor is a configuration that allows an inhibitory substrate to be selectively partitioned between an immiscible and biocompatible organic phase and a cell-containing aqueous phase [1]. By carefully choosing the organic solvent, the substrate partitions into the aqueous phase at a concentration that is not substantially inhibitory to the cells. This system has the advantage of being self-regulatory, in the sense that the xenobiotic substrate is delivered to the aqueous phase in response to the consumption rate of the cells, and the problem of substrate inhibition commonly observed at high concentrations in a batch system is thus effectively eliminated [2].

This two-phase system has been used successfully for the biodegradation of phenol [1–3], pentachlorophenol [4,5], and benzene, toluene and *p*-xylene [6] in batch and fed-batch mode. Previous modelling work on this partitioning bioreactor concept focused on determining optimal phenol feeding strategies for the system operated in fed-batch mode with the objective of maximising phenol degradation within a certain time period [7].

The purpose of the present study was to conduct a preliminary investigation into the operation of the two-phase partitioning bioreactor in continuous mode, with a particular focus on phenol degradation. Continuous operation of the two-phase system has not yet been examined experimentally for any inhibitory substrate, and it was expected that predicting the behaviour of the system with a mathematical model would help establish guidelines and operating conditions for experimental work. Ultimately, this would lead to steady-state operation at the maximum substrate utilisation rate, and would be an advantage over the operation of fed-batch systems, which are time-dependent and, therefore, do not operate continuously at the point of maximum substrate consumption.

A mechanistic model for phenol degradation in a continuously operated two-phase partitioning bioreac-

* Corresponding author. Tel.: +1-613-5332784; fax: +1-613-5336637.

E-mail address: daugulis@chee.queensu.ca (A.J. Daugulis)

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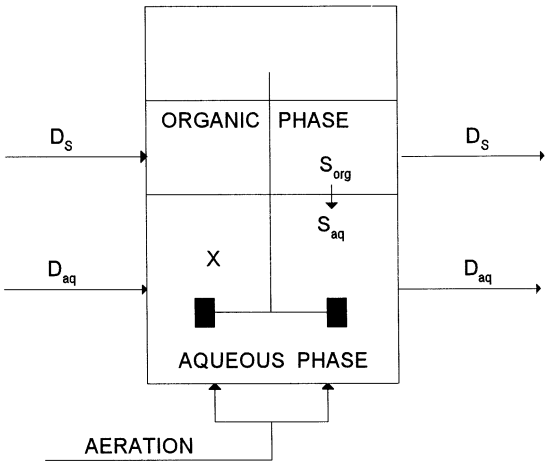

Fig. 1. Schematic view of two-phase partitioning bioreactor in continuous mode.

tor is presented in this paper. Steady-state and dynamic simulations are conducted to predict the behaviour of the system at different operating conditions, as well as to investigate the sensitivity of the bioreactor operation to disturbances in feed phenol concentration.

2. Process description

A schematic view of the two-phase partitioning bioreactor operated in continuous mode is provided in Fig. 1. The set-up is based on the experimental fed-batch two-phase bioreactor described by Collins and Daugulis [1], with the addition of input and output flows. An aqueous feed supplies the necessary, non-carbon nutrients to the cells in the aqueous phase, and the corresponding aqueous flow out provides a bleed stream for the cells. There is also a solvent feed, which supplies the phenol to the organic phase. Based on the experimental set-up, the aqueous and organic phase volumes were assumed to be 1 and 0.5 l, respectively, and the agitation and aeration rates were set at 250 rpm and 0.5 vvm, respectively. The cells used experimentally for phenol degradation were *Pseudomonas putida* ATCC 11172 in an aqueous medium, with 2-undecanone as the immiscible organic phase.

Certain operational challenges were encountered with the system during experimental fed-batch operation, which were believed to be applicable to continuous operation and, therefore, needed to be addressed in the development of the mathematical model. The first operational challenge was biofilm formation, foaming and presence of cells in the organic layer which occurred during active cell growth [2]. When the cell activity in the system increased, the production of CO_2 became greater, which led to a greater entrainment of cells into the organic layer. These entrained cells were not believed to actively participate in phenol consumption. A cell entrainment term was, therefore, included in the model to take this phenomenon into account. Another recurring operational challenge in the system was oxygen limitation during periods of high phenol consumption [3]. With only air being supplied (i.e. no supplemental O_2), it was not possible to provide enough oxygen to the system to prevent the cells from becoming oxygen limited without using aeration rates which would cause excessive foaming and cell and solvent loss. Accordingly, the model also incorporated consideration of oxygen transfer and oxygen-limiting kinetics.

3. Model development

3.1. Key process characteristics

The key process characteristics for this system, including growth kinetics for substrate inhibition, cell entrainment, mass transfer in a two-phase system, and partition coefficient determination, have already been discussed for fed-batch operation of the partitioning bioreactor [7]. These key process characteristics also apply to continuous operation and are not repeated here. In addition, the following simplifying assumptions need to be addressed for continuous mode operation:

1. There is no volume change in the system, i.e. the flow in and the flow out of each phase are constant and equal. This leads to:

$$\frac{dV_{aq}}{dt} = 0 \quad \text{and} \quad \frac{dV_{org}}{dt} = 0 \tag{1}$$

Since no volume change takes place and operation occurs at the nominal volume, then

$$V_{aq} = V_{n,aq} \quad \text{and} \quad V_{org} = V_{n,org} \tag{2}$$

where $V_{n,aq}$ and $V_{n,org}$ are the nominal volumes for the aqueous and organic phases, respectively.

2. There are also no density changes in the aqueous and organic phases, i.e.

$$\frac{d\rho_{aq}}{dt} = 0 \quad \text{and} \quad \frac{d\rho_s}{dt} = 0 \tag{3}$$

In addition, the densities are uniform,

$$\rho_{aq} = \rho_{aq,o} \quad \text{and} \quad \rho_s = \rho_{s,o} \tag{4}$$

where $\rho_{aq,o}$ is the density of the aqueous feed and $\rho_{s,o}$ is the density of the organic feed.

3. The effluent from the organic phase removes only the organic phase, and the effluent from the aqueous phase removes only the aqueous phase.
4. The feed to the aqueous phase is sterile, i.e. no cells are present:

$$X_0 = 0 \text{ g } l^{-1} \tag{5}$$

where X_0 is the feed cell concentration.

Table 1
Model parameters used in this study

Parameter	Value	Unit
k_d	0.001	h^{-1}
k_e	0.57	
K_I	470	mg l^{-1}
$X_L a$	250	h^{-1}
$k_L a_{O_2}$	43	h^{-1}
K_o	0.048	mg l^{-1}
K_s	1.0	mg l^{-1}
V_{aq}	1.0	l
V_{org}	0.5	l
$Y_{O_2/x}$	1/0.338	g/g
$Y_{x/s}$	0.52 ± 0.08	g/g
μ_{max}	0.534	h^{-1}
$C^*_{O_2}$	37.3[a]	mg l^{-1}

[a] Pure oxygen at 30°C.

3.2. Model equations

The mathematical model for the partitioning bioreactor was based on four component balances and two total balances. The variables of interest are the cell concentration in the aqueous phase X, aqueous phase phenol concentration S_{aq}, organic phase phenol concentration S_{org}, and oxygen concentration C_{O_2} in the aqueous phase as a function of time. The two total material balances for the aqueous and organic phases are not required because of the simplifying assumptions above for continuous operation, and thus, the model is reduced to its final form in Eqs. (6)–(9).

$$\frac{dX}{dt} = \mu X - k_d X - k_e \mu X - D_{aq} X \tag{6}$$

$$\frac{dS_{aq}}{dt} = K_L a\left(\frac{S_{org}}{D_{phenol}} - S_{aq}\right) - \frac{\mu X}{Y_{x/s}} - D_{aq} S_{aq} \tag{7}$$

$$\frac{dS_{org}}{dt} = D_s S_{org,o} - K_L a\left(\frac{S_{org}}{D_{phenol}} - S_{aq}\right)\frac{V_{aq}}{V_{org}} - D_s S_{org} \tag{8}$$

$$\frac{dC_{O_2}}{dt} = D_{aq} C_{O_2} + K_L a_{O_2}(C^*_{O_2} - C_{O_2}) - Y_{O_2/x}\mu X - D_{aq} C_{O_2} \tag{9}$$

where μ is the specific growth rate (h^{-1}), k_d the maintenance coefficient (h^{-1}), k_e the entrainment coefficient, D_{aq} the aqueous dilution rate (h^{-1}), D_s the solvent dilution rate (h^{-1}), $K_L a$ the overall mass transfer coefficient for phenol (h^{-1}), D_{phenol} the phenol partition coefficient ((g l^{-1} org)/(g l^{-1} aq)), $Y_{x/s}$ the growth yield coefficient (g/g), $S_{org,o}$ the feed substrate concentration (g l^{-1}), $k_L a_{O_2}$ the mass transfer coefficient for oxygen (h^{-1}), $C^*_{O_2}$ the saturation concentration of oxygen (g l^{-1}), and $Y_{O_2/x}$ is the oxygen yield coefficient (g/g). A volume correction was made for the organic phase phenol concentration in Eq. (8), since the aqueous and organic phase volumes were different (refer to process description), and also to ensure that the units were consistent. The values of the model parameters are listed in Table 1. These parameters are a combination of literature values and estimates from existing experimental data [7].

In the above model, μ is based on the Seker et al. model [8], derived for dual-substrate growth: Haldane kinetics for phenol and Monod kinetics for oxygen,

$$\mu = \frac{\mu_{max} S_{aq}}{K_s + S_{aq} + (S^2_{aq}/K_I)} \frac{C_{O_2}}{K_O + C_{O_2}} \tag{10}$$

where μ_{max} is the maximum specific growth rate (h^{-1}), K_s the substrate saturation constant (g l^{-1}), K_I the substrate inhibition constant (g l^{-1}), and K_O is the oxygen saturation constant (g l^{-1}).

The partition coefficient of phenol between the solvent and the aqueous growth medium was determined experimentally [7] and fitted to the following equation.

$$D_{phenol} = 1.215(9.75\exp[-1.8182 S_{org}] - 48.75\exp[-6.6667 S_{org}] + 39.0) \tag{11}$$

3.3. Model assumptions

The general assumptions made in the development of this model are as follows, (1) the solvent is biocompatible and immiscible with the aqueous medium so that cell growth is not adversely affected; (2) there is no solvent loss due to consumption by the cells, i.e. the solvent is not used as a carbon source; (3) the two individual phases (organic and aqueous) are well-mixed so that no stagnant regions exist in the bioreactor and each phase is considered homogenous, with no emulsions being formed; (4) the non-carbon nutrients (not including oxygen) are supplied in excess so that they do not become limiting during the course of the fermentation; (5) the only products formed are cells and CO_2 with no intermediates formed that affect the fermentation; (6) there is no phenol loss to volatilisation; and (7) all phenol consumption occurs in the aqueous phase. These assumptions are considered to be reasonable approximations to the experimental system, and are supported by experimental experience in our laboratory for fed-batch operation of the partitioning bioreactor. Indeed, an analogous fed-batch model form was validated against two sets of existing fed-batch experimental data and good predictions were observed for the time frames of the simulations, as well as trends in the state variables [7].

4. Modelling substrate inhibition in continuous mode

In should be noted that process instability may result when degrading inhibitory substrates in conventional continuous culture operation [9], since there is the

possibility of operating the CSTR at three steady-states [10]. Two of these steady states were typically stable and the third was typically unstable. Disturbances in operating variables, particularly changes in dilution rate and/or substrate concentration in the feed, may cause washout of the biomass from the bioreactor or transient increase in the substrate concentration by perturbing operation from the steady-state point [11].

When the two-phase partitioning bioreactor was operated in fed-batch mode, only two variables were available for process manipulation, the amount of phenol added to the organic phase, and the time(s) at which this addition occurred. In continuous operation of the two phase system, however, there were three variables available for process manipulation, the feed substrate concentration $S_{org,o}$, the aqueous dilution rate D_{aq}, and the solvent dilution rate D_s. Different sets of operating conditions for the two-phase partitioning bioreactor have been investigated based on these three manipulated variables.

4.1. Results

4.1.1. Steady-state simulations

The first investigation into continuous operation of the partitioning bioreactor was intended to determine the percent removal of organic phase phenol in the feed based on three representative pairs of values for the solvent dilution rate and phenol feed concentration. These pairs were chosen to cover a wide range of possible values for D_s and $S_{org,o}$. The steady-state model was obtained by setting all time derivatives to zero in Eqs. (6)–(9). This resulted in a system of non-linear algebraic equations, which was solved using a numerical method based on successive substitution in the symbolic computation package Maple [12]. The results are shown in Fig. 2.

The shapes of the curves in Fig. 2 suggest that there is the possibility of operating at two different percent removal values for a given value of D_{aq} within certain ranges. This phenomenon arises from the fact that in a steady-state of a chemostat culture, the growth rate μ, (defined by the Seker et al. [8] model of Eq. (10) in this instance), can be equated with the dilution rate D_{aq} [13]. The quadratic form of the Haldane equation, which describes phenol degradation in the Seker et al. model, indicates that there is the possibility of operating at two different steady-states for the cell and substrate concentrations at given specific growth rates and, therefore, at given dilution rates [9]. Previous work on substrate inhibited systems has shown that one of these steady states was stable and the other was unstable [13]. There is actually a third steady state at each D_{aq} corresponding to washout conditions, i.e. when the cell concentration is zero (not shown in Fig. 2). Typically, under washout conditions the percent removal rate of substrate is zero. However, in the two-phase bioreactor, the percent removal of organic phase phenol under washout conditions was not zero, since some phenol did partition into the aqueous phase. As the aqueous dilution rate increased, the amount of phenol that partitions into the aqueous phase increased.

The result from Fig. 2 indicates that there is also the possibility of having three different cell concentrations for fixed values of aqueous dilution rates. The cell concentrations corresponding to the three pairs of D_s and $S_{org,o}$ are plotted in Fig. 3. As expected, between points I and II, there are two possible steady-state cell concentrations for each D_{aq}. There is also a trivial solution to the steady-state cell balance in Eq. (6),

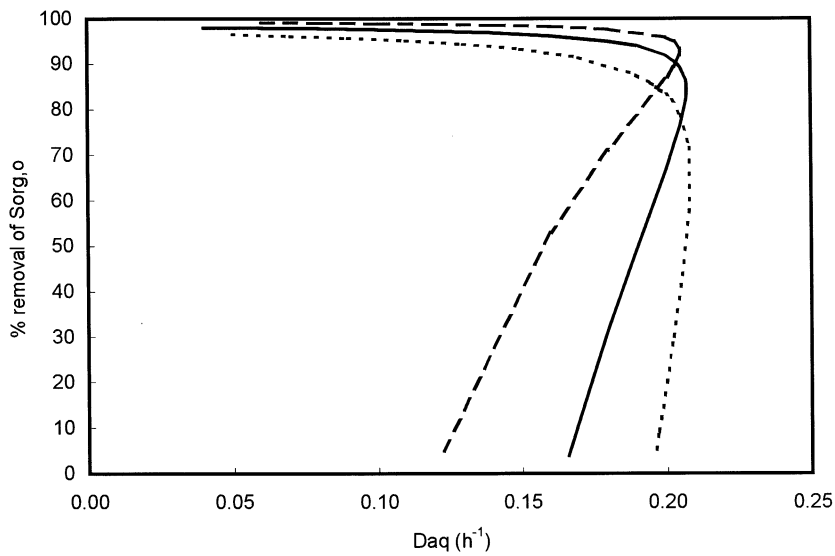

Fig. 2. Percent removal of $S_{org,o}$ in a simulated, two-phase partitioning bioreactor operated in continuous mode when (a) $D_s = 0.105\ h^{-1}$ and $S_{org,o} = 20.0\ g\ l^{-1}$ (– –); (b) $D_s = 0.25\ h^{-1}$ and $S_{org,o} = 8.5\ g\ l^{-1}$ (—); and (c) $D_s = 0.55\ h^{-1}$ and $S_{org,o} = 3.5$ (- - -).

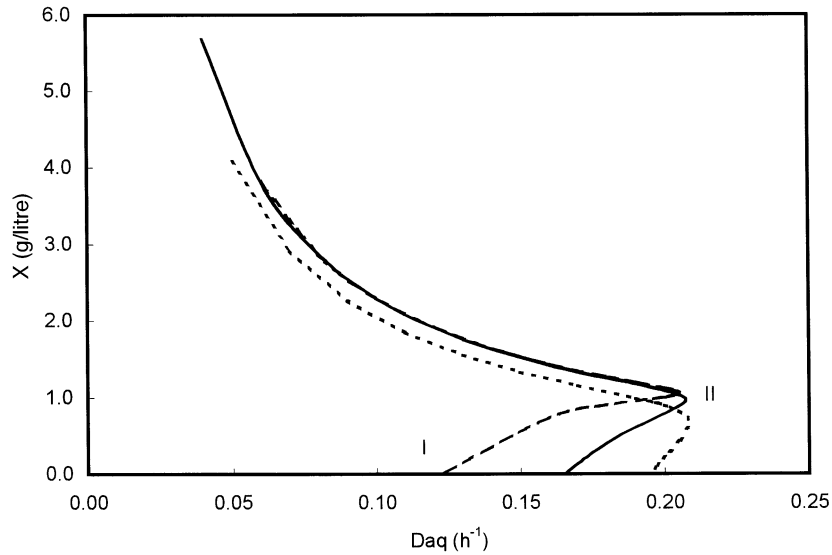

Fig. 3. Cell concentration in a simulated, two-phase partitioning bioreactor operated in continuous mode when (a) $D_s = 0.105\ h^{-1}$ and $S_{org,o} = 20.0\ g\ l^{-1}$ (– –); (b) $D_s = 0.25\ h^{-1}$ and $S_{org,o} = 8.5\ g\ l^{-1}$ (—); and (c) $D_s = 0.55\ h^{-1}$ and $S_{org,o} = 3.5$ (- - -).

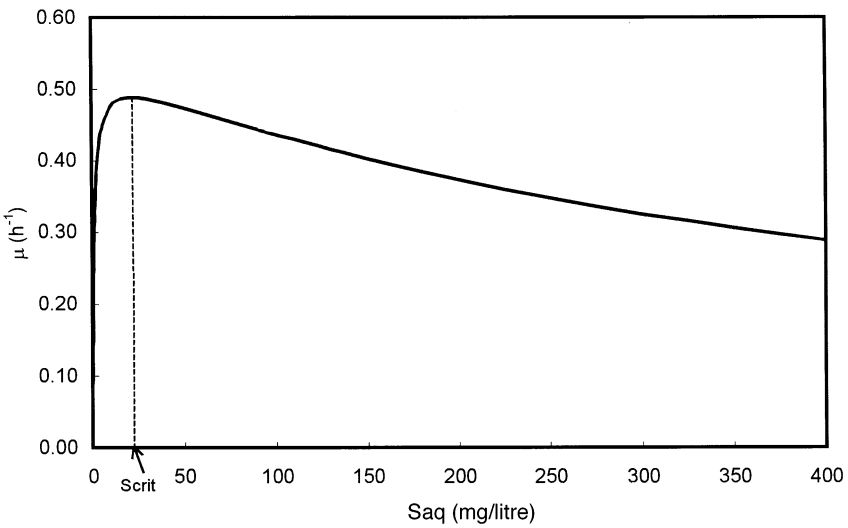

Fig. 4. Specific growth rate curve for substrate inhibition using the Seker et al. model [8] with $\mu_{max} = 0.534\ h^{-1}$, $K_s = 1\ mg\ l^{-1}$, $K_I = 470\ mg\ l^{-1}$, $K_O = 0.048\ mg\ l^{-1}$, and constant $C_{O_2} = 37.3\ mg\ l^{-1}$.

corresponding to the washout cell concentration ($X = 0$ g l^{-1}) at each aqueous dilution rate, which is not shown in Fig. 3.

For aqueous dilution rates at which multiple steady states occur in Figs. 2 and 3, if $S_{aq} < S_{crit}$ (where S_{crit} is defined as the substrate concentration at the maximum specific growth rate in the presence of inhibition, as shown in Fig. 4), the growth rate will increase in response to an increase in S_{aq}. In other words, the culture is self-regulating and the steady state is stable. However, if $S_{aq} > S_{crit}$, the steady state will not be stable, because an increase in S_{aq} will decrease the growth rate so that the culture will be more inhibited, which will result in an increase in S_{aq}. The increased S_{aq} will again decrease growth rate, and this situation will continue, ultimately leading to washout. Conversely, if a disturbance decreases S_{aq}, when $S_{aq} > S_{crit}$, the growth rate will increase and the biomass will increase until it comes to a stable steady-state value [13].

The stable steady-state cell concentrations in Fig. 3 are represented by the higher cell concentrations (corresponding to $S_{aq} < S_{crit}$) for each pair of D_s and $S_{org,o}$. These stable steady-state concentrations are located on the upper branch of each curve. The unstable steady-states are represented by the lower cell concentrations (corresponding to $S_{aq} > S_{crit}$) between points I and II, which can be thought of as the middle branch. There is also a washout branch, which is not shown in Fig. 3.

One question that arises after examining Figs. 2 and 3 is whether there is any advantage of operating at low

D_s and high $S_{org,o}$ versus operating at high D_s and low $S_{org,o}$ to achieve the same extent of phenol biodegradation. As can be seen from these figures, if the bioreactor is operated at low D_s values, then there is a wider range of aqueous dilution rates over which there are two possible non-washout steady-states (from $D_{aq} \sim 0.12$ to $D_{aq} \sim 0.205\ h^{-1}$). On the other hand, if the bioreactor is operated at high D_s values, the range of D_{aq} giving two possible steady-states is much smaller. This may influence the sensitivity of the system to disturbances in the feed phenol concentration, or influence the length of time required to reach another steady state. These questions are considered next in the dynamic investigations.

4.1.2. Dynamic simulations

Further investigations into the unstable nature of the steady states in the two-phase partitioning bioreactor were considered for two operating conditions using dynamic simulations at the following conditions, (1) $D_s = 0.105\ h^{-1}$, $S_{org,o} = 20\ g\ l^{-1}$ and $D_{aq} = 0.15\ h^{-1}$; and (2) $D_s = 0.55\ h^{-1}$, $S_{org,o} = 3.5\ g\ l^{-1}$, and $D_{aq} = 0.205\ h^{-1}$. These operating conditions, corresponding to approximately 40% removal of phenol at unstable steady states for curves (a) and (c) in Fig. 2. These two sets of operating conditions were selected from the results of Figs. 2 and 3, since the shapes of their curves were substantially different. It was believed that these different shapes could potentially reflect the sensitivity of the system to disturbances in phenol feed concentration. The effect of a disturbance in the feed concentration $S_{org,o}$ on the system was investigated by performing dynamic simulations in Matlab®, using the ode15s subroutine for stiff differential equations [15].

4.1.2.1. Low solvent dilution rate and high feed substrate concentration. The unstable nature of the steady state (i.e. the steady state located in the lower branch) for the case of high feed substrate concentration and low solvent dilution rate is shown in Fig. 5 for a 1% (or 0.2 g l^{-1}) step change in $S_{org,o}$. Two scenarios are represented in this figure. When a step decrease in the phenol feed concentration occurs, the system responds, with an increase in X and a decrease in S_{org}. The system moves from the unstable steady state in the middle branch to a stable steady state in the upper branch area. The new steady state is not directly located on the curves of Figs. 2 and 3, since for a sustained perturbation in the feed phenol concentration, slightly different steady-state cell and phenol profiles are generated. The system stabilises after approximately 125 h or 13.1 reactor volumes in the organic phase and 18.8 reactor volumes in the aqueous phase.

However, when a step increase in the phenol feed concentration occurs, the system washes out, i.e. the cell concentration goes to zero in approximately 400 h. These two responses confirm that this steady-state is indeed unstable. That is, the system responds by moving from the unstable middle branch to the washout branch on the upper branch. Similar results for the unstable steady state of a single phase-continuous culture utilising an inhibitory substrate have been obtained [9].

A simulation was also carried out starting at the stable state for the same conditions. Step increases and step decreases of 0.2 g l^{-1} in $S_{org,o}$ were made and the system quickly responded by moving to a new steady state position on the upper branch, indicating that the steady state was indeed stable.

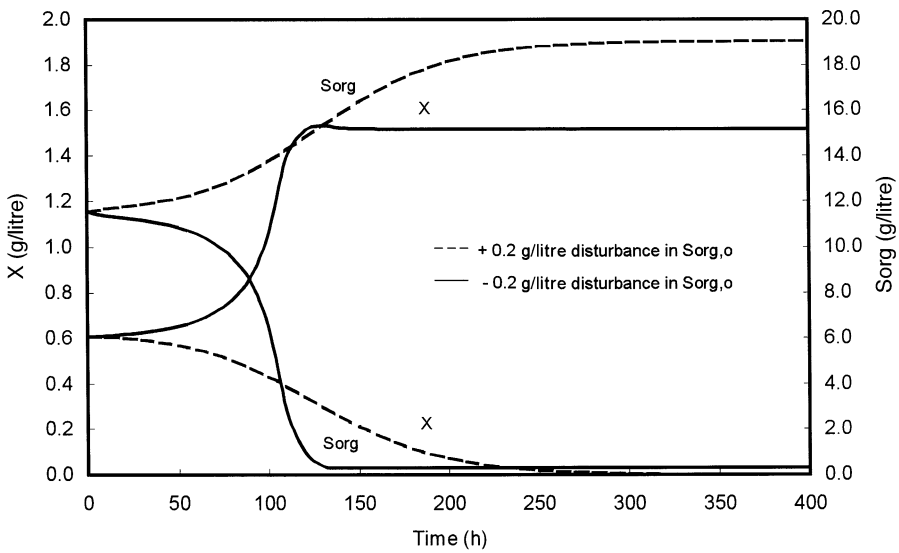

Fig. 5. Simulated state trajectories for a two-phase partitioning bioreactor operated in continuous mode at $S_{org,o} = 20\ g\ l^{-1}$, $D_{aq} = 0.15\ h^{-1}$ and $D_s = 0.105\ h^{-1}$ with a 0.2 g l^{-1} disturbance in $S_{org,o}$.

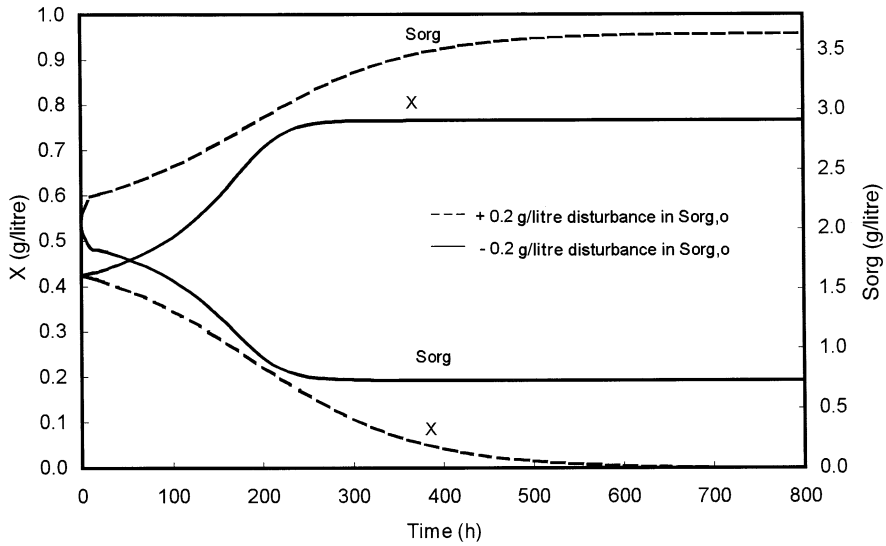

Fig. 6. Simulated state trajectories for a two-phase partitioning bioreactor operated in continuous mode at $S_{org,o} = 3.5$ g l^{-1}, $D_{aq} = 0.205$ h^{-1} and $D_s = 0.55$ h^{-1} with a 0.2 g l^{-1} disturbance in $S_{org,o}$.

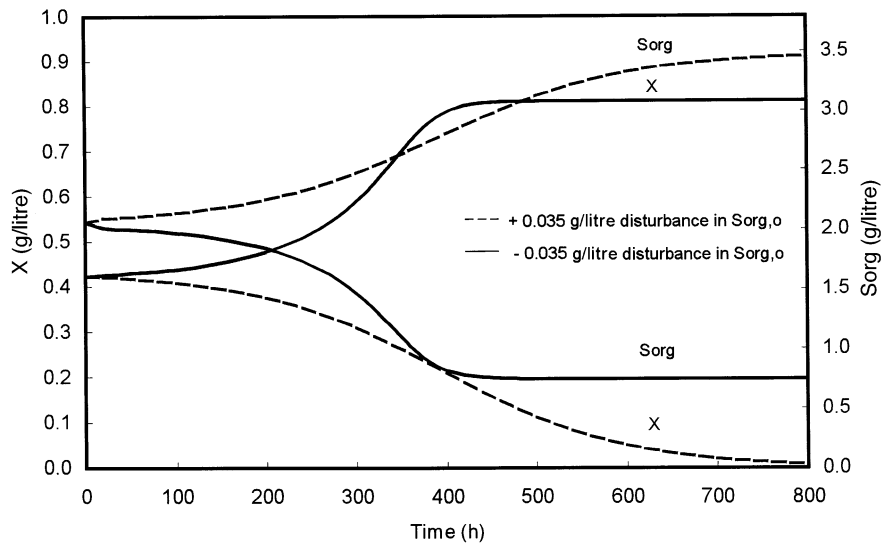

Fig. 7. Simulated state trajectories for a two-phase partitioning bioreactor operated in continuous mode at $S_{org,o} = 3.5$ g l^{-1}, $D_{aq} = 0.205$ h^{-1} and $D_s = 0.55$ h^{-1} with a 0.035 g l^{-1} disturbance in $S_{org,o}$.

4.1.2.2. High solvent dilution rate and low feed substrate concentration. The unstable nature of the steady state was also investigated at low feed substrate concentration and high solvent dilution rate. Two cases were considered. In the first case, a disturbance of the same size (0.2 g l^{-1}) as for the low solvent dilution rate and high feed substrate concentration study was made. In the second case, a disturbance of 1% (or 0.035 g l^{-1}) in the feed substrate concentration was made. The results are presented in Figs. 6 and 7.

In both the cases, when a step decrease in $S_{org,o}$ was made, the system moved to a stable steady state in the upper branch. When a step increase in $S_{org,o}$ was made, the system washed out.

For the case of a 0.2 g l^{-1} step decrease in the feed substrate concentration, the system reached a stable state in the upper branch in approximately 250 h, corresponding to 137.5 reactor volumes in the organic phase, and 51.3 reactor volumes in the aqueous phase. This was considerably longer than for the case presented in Fig. 5, which was for a low D_s and a high $S_{org,o}$. One reason for this could be that the steady-state cell concentration at the initial conditions for the latter test (shown in Fig. 6) is smaller than that in the first scenario (shown in Fig. 5), and, therefore, the system takes longer to respond to a disturbance in $S_{org,o}$.

For the case of 1% (or 0.035 g l^{-1}) step decrease in the feed concentration, the reactor reached a stable

state in the upper branch in 400 h corresponding to 220 reactor volumes in the organic phase and 82 reactor volumes in the aqueous phase. Again this is much longer than the results presented in Fig. 5. The time to reach the stable steady state was also much longer than for 0.2 g l^{-1} disturbance in the feed concentration, as seen in Fig. 6. This indicates that if a disturbance of small magnitude entered the system, it would take longer for the system to reach its new steady state or to wash out. Thus, the effective time constant of this system was not constant, and depended on the disturbance magnitude and region of operation. This is a manifestation of the non-linearity in the process dynamics.

Physically this can be understood by considering where the unstable steady-state is located on the μ versus S_{aq} curve (shown in Fig. 4). For all runs in which $S_{aq} > S_{crit}$, a larger step decrease in S_{aq} (as a result of a step decrease in $S_{org,o}$) will cause the growth rate to increase more rapidly (i.e. move up the μ curve) and, therefore, this will result in the cells growing more quickly and reaching a stable steady state faster. If a larger step increase in S_{aq} occurs, this will result in greater substrate inhibition (i.e. move 'down' the μ curve) and the cells are likely to wash out more quickly. Similar results have been obtained for a one-phase continuous culture with substrate inhibition [14].

The results from the dynamic simulations can be applied to the steady state operating regions in Fig. 3. When the unstable steady-state cell concentration in the middle branch for the low D_s and high $S_{org,o}$ operating region (curve a) is perturbed by a disturbance in the feed concentration, the system will respond quickly, either reaching the new stable steady state or washing out as demonstrated in Fig. 5. However, when considering operation at the unstable steady state for the high D_s and low $S_{org,o}$ region (curve c), the system was not as sensitive to a disturbance in the feed concentration, and it would take longer for the system to reach the stable steady state or to wash out.

The question of the 'ideal' operating region should be addressed next. Ultimately, operation at a stable steady state is desired, since high removal rates of phenol were achieved and the system was stable in the presence of fluctuating feed concentrations. However, in the event that the system is operated at the unstable state in the middle branch, the question arises as to whether operation at low solvent dilution rate and high feed substrate concentration, or high solvent dilution rate and low feed substrate concentration would be preferable. As already mentioned, if the system operates at low D_s and high $S_{org,o}$ it will respond more quickly to a disturbance in the feed. This is desired if the objective was to reach a stable steady state in the upper branch starting at the unstable steady state. However, as a tradeoff, there is also a possibility of washing out more quickly. If the operating condition were at high D_s and low $S_{org,o}$, then there would be more time available to try and prevent the system from washing out in the event of a disturbance, but at the same time, it would take longer to reach the stable steady state. Obviously, there are benefits and tradeoffs to operating in these two regions and it could be that other factors, such as the type of instrumentation and control, and economic considerations, will be the determining factors in deciding which operating conditions are better.

The dynamic model has, therefore, helped establish the following guidelines for continuous operation of the two-phase partitioning bioreactor.

1. As a first choice, the system should be operated at the stable steady state of any of the three operating conditions shown in Figs. 2 and 3 to achieve high removal of phenol, and to be insensitive to disturbances in operating parameters.

   However, if operation at the unstable steady state is required, then
2. Operate at low D_s and high $S_{org,o}$ if a quick response to changes in feed phenol concentration is required.
3. Operate at high D_s and low $S_{org,o}$ if a slow response to changes in feed phenol concentration is required.
4. Carry out step decreases in $S_{org,o}$ to reach a stable steady state in the upper branch.
5. Carry out step decreases in $S_{org,o}$ of large magnitude to reach the stable steady state more quickly.

5. Conclusions

A dynamic model for continuous operation of the two-phase partitioning bioreactor was developed and presented in this paper, with particular focus on phenol degradation. This model was used to predict the behaviour of the system under different steady-state operating conditions and dynamic disturbances. It was shown that operating the bioreactor at different pairs of values of D_s and $S_{org,o}$ influenced the range of aqueous dilution rates at which multiple steady states occurred, and also had an effect on how sensitive the bioreactor was to disturbances in feed phenol concentration. It was also confirmed that when dealing with substrate inhibition in two-phase systems, there is a possibility of obtaining three steady states for the cell, aqueous phase phenol and organic phase phenol concentrations over given ranges of aqueous dilution rates. One of these points is a trivial steady-state solution representing washout conditions. Of the two non-trivial steady states observed when substrate inhibition was present, the steady state at low cell concentration and high feed substrate concentration appeared to be unstable (i.e. the system washed out) when disturbances in the form of step increases in the feed substrate concentration

were introduced. However, when a step decrease in the feed concentration was made from this unstable steady state, the system recovered by reaching a stable steady state in the upper branch. The results from the dynamic investigations also demonstrated that small disturbances in the feed concentration result in longer transients as a result of the location of the feed substrate concentration disturbance on the specific growth rate curve.

6. Nomenclature

C_{O_2} oxygen concentration in aqueous phase (g l^{-1})
$C^*_{O_2}$ saturation concentration of oxygen (g l^{-1})
D_{aq} aqueous dilution rate (h^{-1})
D_{pheno} phenol equilibrium partition coefficient (g l^{-1} $phenol_{org}$/g l^{-1} $phenol_{aq}$)
D_s solvent dilution rate (h^{-1})
k_d maintenance coefficient (h^{-1})
k_e cell entrainment coefficient
K_La overall mass transfer coefficient for phenol (h^{-1})
$k_La_{O_2}$ mass transfer coefficient for O2 (h^{-1})
K_I substrate inhibition constant (g l^{-1})
K_S substrate saturation constant (g l^{-1})
K_O oxygen saturation constant (g l^{-1})
S_{aq} substrate concentration in aqueous phase (g l^{-1})
S_{crit} substrate concentration at maximum specific growth rate in the presence of inhibition (g l^{-1})
S_{org} substrate concentration in organic phase (g l^{-1})
$S_{org,o}$ substrate concentration added to organic phase (g l^{-1})
t time (h)
V_{aq} volume of aqueous phase (l)
$V_{n,aq}$ nominal volume of aqueous phase (l)
$V_{n,org}$ nominal volume of organic phase (l)
V_{org} volume of organic phase (l)
X aqueous phase cell concentration (g l^{-1})
X_0 feed cell concentration (g l^{-1})
$Y_{O_2/x}$ oxygen consumption coefficient (g/g)
$Y_{x/s}$ cell yield coefficient (g/g)

Greek letters

μ specific growth rate (h^{-1})
μ_{max} maximum specific growth rate (h^{-1})
ρ_{aq} density of aqueous phase (g l^{-1})
$\rho_{aq,o}$ density of aqueous feed (g l^{-1})
ρ_s density of organic phase (g l^{-1})
$\rho_{s,o}$ density of organic feed (g l^{-1})

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